

Amendments to the Claims

This listing of claims will replace all prior versions and listings of all claims in the application.

Claims 1-49 (cancelled)

Please add the following new claims:

50. (New) A non-naturally occurring variant TNF- α protein comprising an amino acid sequence comprising an amino acid substitution as compared to SEQ ID NO:2, said substitution at a position selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140, wherein said variant protein interacts with a naturally occurring human TNF- α to form mixed trimers that have a reduced capacity to effect TNF- α receptor signaling in a caspase activity assay.

51. (New) A variant protein according to claim 50 wherein said variant protein comprises 2 substitutions at positions selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140.

52. (New) A variant protein according to claim 50 wherein said variant protein comprises 3 substitutions at positions selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140.

53. (New) A variant protein according to claim 50 wherein said variant protein comprises 4 substitutions at positions selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140.

54. (New) A variant protein according to claim 50 wherein said variant protein comprises 5 substitutions at positions selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140.

55. (New) A variant protein according to claim 1 comprising a covalent modification.

56. (New) A variant protein according to claim 55 wherein said covalent modification comprises a polyethylene glycol molecule.

57. (New) A variant protein according to claim 50 further comprises a substitution at position 145.

58. (New) A TNF- α variant protein comprising an amino acid sequence comprising an amino acid substitution as compared to SEQ ID NO:2, said substitution selected from the group consisting of Q21R, Q21K, N30E, R31V, R31L, N30D, R31I, R31D, R32D, R32E, R32S, R32H, R32T, A35T,

A35S, G66Q, G66K, G66N, G66R, G66E, A111R, A111E, A111K, A111D, K112D, K112E, Y115Q, Y115K, Y115E, Y115N, Y115R, Y115F, Y115H, Y115M, Y115L, Y115I, Y115D, Y115T, Y115S, Y115V, D140R, D140K, D140Q, D140E, F144Q, F144H, F144N, E146N, E146K, E146D, E146K, E146Q, E146H, E146E, E146T and E146S, wherein said variant protein interacts with a naturally occurring human TNF- α to form mixed trimers that have a reduced capacity to effect TNF- α receptor signaling in a caspase activity assay.

59. (New) A method of forming mixed TNF- α trimers comprising combining:

a) a first variant TNF- α trimer comprising a non-naturally occurring variant TNF- α protein comprising an amino acid sequence having an amino acid substitution as compared to SEQ ID NO:2, said substitution at a position selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140; and

b) a naturally occurring human TNF- α trimer;

under conditions whereby mixed trimers are formed that have a reduced capacity to effect TNF- α receptor signaling in a caspase activity assay.

60. (New) A method according to claim 59 wherein said first variant trimer has two variant monomers.

61. (New) A method according to claim 60 wherein said first variant trimer has three variant monomers.

62. (New) A method according to claim 59 wherein said variant protein comprises a covalent modification.

63. (New) A method according to claim 62 wherein said covalent modification comprises a polyethylene glycol molecule.

64. (New) A TNF- α mixed trimer comprising a variant TNF- α protein comprising an amino acid sequence having an amino acid substitution as compared to SEQ ID NO:2, said substitution at a position selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140, wherein said mixed trimers have a reduced capacity to effect TNF- α receptor signaling in a caspase activity assay.

65. (New) A mixed trimer according to claim 64 wherein one of the monomers in said trimer is a human TNF- α monomer.

66. (New) A mixed trimer according to claim 64 wherein two of the monomers in said trimer are independently selected variant proteins.

67. (New) A mixed trimer according to claim 66 wherein said variant proteins are the same.

68. (New) A mixed trimer according to claim 66 wherein said variant proteins are different.

69. (New) A mixed trimer according to claim 64 where all of the monomers in said trimer are variant proteins.

70. (New) A TNF- α mixed trimer comprising a variant TNF- α monomer comprising an amino acid sequence having an amino acid substitution as compared to SEQ ID NO:2, wherein said substitution is selected from the group consisting of K112D, Y115T, Y115I, D143K and D143R.

71. (New) A mixed trimer according to 70 further comprising a second variant monomer having an amino acid sequence with an amino acid substitution as compared to SEQ ID NO:2.

72. (New) A TNF- α mixed trimer comprising a variant TNF- α protein comprising an amino acid substitution as compared to SEQ ID NO:2, wherein said substitution is selected from the group consisting of Q21R, Q21K, N30E, R31V, R31L, N30D, R31I, R31D, R32D, R32E, R32S, R32H, R32T, A35T, A35S, G66Q, G66K, G66N, G66R, G66E, A111R, A111E, A111K, A111D, K112D, K112E, Y115Q, Y115K, Y115E, Y115N, Y115R, Y115F, Y115H, Y115M, Y115L, Y115I, Y115D, Y115T, Y115S, Y115V, D140R, D140K, D140Q, D140E, F144Q, F144H, F144N, E146N, E146K, E146D, E146K, E146Q, E146H, E146E, E146T and E146S.